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HED File Code	**13000 Tox Reviews**
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CASWELL FILE

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OPP OFFICIAL RECORD
HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

JUN 9 - 1993

010314

MEMORANDUM

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

SUBJECT: Methiocarb (Mesurol):

1. Review of Supplemental Information (MRID 424810-01) Submitted for a 21-Day Dermal Study with Mesurol Technical in Albino Rabbits (MRID 417717-01);

2. Establishment of a NOEL/LOEL for 21-Day Dermal Toxicity Testing with Mesurol Technical (Guideline Series 82-2).

Barcode: D183114
Submission: S426737
PC Code: 100501
Tox Chem: 578B

TO: Karen Farmer/Linda Probst
PM Team #73
Reregistration Division

FROM: Karen L. Hamernik, Ph.D., Pharmacologist
Section Head, Section 3
Toxicology Branch I
Health Effects Division (H7509C)

K. Hamernik 6/7/93

THRU: Karl Baetcke, Ph.D.
Chief, Toxicology Branch I
Health Effects Division (H7509C)

Karl D. Baetcke 6/9/93

CONCLUSIONS

1. The result of a review of a Supplemental Submission to MRID 417717-01, a 21-Day Dermal Toxicity Study in Rabbits with Mesurol technical, is as follows:

Although all the data requested were submitted and were found to satisfactorily address questions asked in the Data Evaluation Report (DER), the study cannot be upgraded from the Supplemental category because clear cut signs of toxicity were not observed at the highest dose tested of 500 mg/kg/day and this was less than the limit dose of 1000 mg/kg/day.

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2. When all of the 21-day dermal toxicity data submitted for Mesurol technical are taken together, a LOEL for subchronic dermal toxicity can be established at 250 mg/kg/day based on mortality in females at doses of 250 mg/kg/day and above in a dose-ranging study and a NOEL can be established at 150 mg/kg/day which is the next lower dose tested from amongst the studies in which no treatment-related effects were noted. The combined data can be used to fulfill the requirement for a 21-day dermal toxicity study with the technical material.

3. The sponsor should be reminded to submit the additional information requested by the Agency for the rabbit 21-day dermal toxicity study MRID 409223-01 (i.e. individual animal data for clinical observations, a signed statement certifying test material purity, and a description of the methodology used to measure cholinesterase activity) since it will be used in combination with the other 21-day dermal toxicity data submitted by the sponsor to fulfill Guideline Series 82-2 requirements.

ACTION REQUESTED

Review supplemental information (MRID 424810-01) provided by the sponsor to address questions in a previous review of a 21-day dermal toxicity study with Mesurol technical (MRID 417717-01).

TOXICOLOGY BRANCH EVALUATION

1. Review of Supplemental Information (MRID 424810-01) Submitted for a 21-Day Dermal Study with Mesurol Technical in Albino Rabbits (MRID 417717-01).

Background. In the review of a rabbit 21-day dermal toxicity study (see memo of J. Redden, dated 9/13/91, D163753), additional information was requested of the sponsor, Miles, Inc. (formerly Mobay, Corp). The review requested that the purity of the test material, individual animal clinical signs data, individual animal back-shaving dates, and information on the method used to measure cholinesterase activity be submitted since this information had not been included in the original report.

Review of Company Response. The sponsor submitted all of the requested information (Report number 98369-2, dated 8/20/92). The purity of the test material (Mesurol technical, Batch No. 86I004), as listed on the signed data analysis sheet, was 98.9%. Cholinesterase activity was measured using a modified Ellman method. The methodology information submitted is attached to this memo.

The individual animal clinical sign data provided did not reveal anything that would contradict the conclusions in J. Redden's review. Dermal irritation was noted mostly towards the end of the

study in some of the treated animals but it is difficult to tell if any of it was treatment-related because control animals also had similar signs of dermal irritation and some of the findings observed in both control and Mesurol-treatment groups may have been due to or exacerbated by repeated shaving of the application site. [Backs were shaved the day before study initiation, and on study days 9, 16, 19, and 21].

Toxicology Branch Conclusion. The J. Redden review concluded that neither a dose high enough to elicit a definitive systemic toxicological response nor the limit dose of 1000 mg/kg/day was administered in the study. Therefore, the study, in and of itself, did not fulfill the requirements for a 21-day dermal toxicity study under Guideline Series 82-2.

There is nothing in the sponsor's amendment that would alter this conclusion. Thus, the study is still classified as Core Supplemental data.

2. Establishment of a NOEL/LOEL for 21-Day Dermal Toxicity Testing with Mesurol Technical.

Background. To date, the sponsor has submitted reports for four 21-day dermal toxicity studies with Mesurol technical, all of which have been reviewed by Toxicology Branch I and all of which were classified as Core Supplemental data. Two of these, a six day initial range-finding study in rabbits[1] and a 21-day repeated dermal toxicity study in rabbits [interrupted at day 14][2] were performed by the same laboratory, Microbiological Associates, Inc.

In the dose-ranging study[1], doses of 0 (saline vehicle), 15.7, 31.3, 62.5, 125, 250, 500, 1000, or 2000 mg/kg/day of Mesurol technical were administered. Death occurred in one out of two females per group at doses of 250, 500, and 1000 mg/kg/day and in both of the high dose females. One out of two males per group died at doses of 1000 mg/kg/day and above. All deaths were ascribed to treatment since no other explanation was provided in the study report. Other clear-cut signs of toxicity (tremors, respiratory difficulties, diarrhea, excess salivation, and lethargy) were noted at the top two doses. No effects were reported at doses of 125 mg/kg/day and below for either sex (two animals per sex per group).

The interrupted 21-day study[2], in which doses of 0 (saline vehicle), 60, 150, or 375 mg/kg/day of Mesurol technical were being administered was stopped due to three accidental deaths at the high dose. No treatment-related effects were observed by day 14 at the lower doses.

Two rabbit 21-day dermal toxicity studies[3,4], which were performed at later dates by a different laboratory, Bio-Research Laboratories, Ltd., Senneville, Quebec, were also submitted. In the first of these studies (report issued 11/23/88), Mesurol technical was administered at doses of 0 (saline vehicle), 60, 150, or 375 mg/kg/day and, in the second study (report issued 8/31/89), Mesurol technical was administered at doses of 0 (saline vehicle) or 500 mg/kg/day. It was for this second study that the supplemental information reviewed in (1.) above was submitted.

There are no clear adverse findings in these studies[3,4], particularly when they are looked at together, thus a convincing LOEL cannot be established using these studies as a basis.

Toxicology Branch Conclusion. Although inconsistencies are noted in the data from the two laboratories and the quality of the data from the two completed 21-day dermal studies[3,4] from Bio-Research Labs exceeds that of the earlier studies from Microbiological Assoc.[1,2], the deaths in the dose ranging study cannot be ignored. Therefore, when all the data are taken together, the LOEL for subchronic dermal toxicity is 250 mg/kg/day based on mortality in females at doses of 250 mg/kg/day and above in the dose-ranging study and the NOEL is 150 mg/kg/day (the next lower dose tested from amongst the studies in which no treatment-related effects were noted).

REFERENCES

1 Six Day Initial Range-Finding Study for a 21-Day Repeated Dermal Toxicity Study (Rabbits) [and Extension], Microbiological, Assoc., performed in January and February, 1988, no MRID, no report number, data were submitted by Mobay as a study progress letter on 4/11/88, EPA Record No.220720 which was reviewed in a memo from K. Hamernik to M. Mautz, dated 2/28/92 (HED document 9324).

2. 21-Day Repeated Dermal Toxicity Study (Rabbits)[Interrupted at Day 14]. Microbiological Assoc., Inc. (MBA Study G-7081.132), performed from 3/9/88 until interruption on 3/22/88, no MRID, data were submitted by Mobay as a study progress letter on 4/11/88, EPA Record No.220720 and was reviewed in a memo from K. Hamernik to M. Mautz, dated 2/28/92 (HED document 9324).

3. A 21-day Dermal Toxicity Study of Mesurol Technical in Albino Rabbits, Bio-Research Laboratories, Ltd., Senneville, Quebec, MRID 409223-01, Study Number 51901, report issued 11/23/88, and reviewed in a memo from K. Hamernik to L. Probst/K.Farmer, under D176010.

010314

4. A 21-day Dermal Toxicity Study of Mesurol Technical in Albino Rabbits, Bio-Research Laboratories, Ltd., Senneville, Quebec, MRID 417717-01, Study Number 51925, report issued 8/31/89, and reviewed in a memo from J. Redden to N. Tompkins, dated 9/13/91, under D163753, S394833).

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Page _____ is not included in this copy.

Pages 7 through 13 are not included in this copy.

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FINAL

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DATA EVALUATION REPORT

MESUROL® Technical

Study Type:
Repeated Dose Dermal Toxicity: 21-Day Study

Study Title:
A 21-Day Dermal Toxicity Study of MESUROL® Technical in Albino Rabbits

Prepared for:

Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by:

Clement International Corporation
9300 Lee Highway
Fairfax, VA 22031-1207

Principal Reviewer Sara Lundgaard Date 6/3/93
Sara Lundgaard, M.S.

Independent Reviewer John Liccione Date 6/3/93
John Liccione, Ph.D.

QA/QC Manager Sharon J. Segal Date 6/3/93
Sharon Segal, Ph.D.

Contract Number: 68D10075
Clement Number: 19
Work Assignment Number: 2-08.1
Project Officer: Caroline Gordon

Guideline Series 82-2:
21-Day Dermal Toxicity Study in Rabbits

EPA Section Head and
Reviewer: Karen Hamernik
Review Section III, Toxicology Branch I,
Health Effects Division

Signature: Karen Hamernik
Date: 6/7/93

DATA EVALUATION REPORT

STUDY TYPE: 21-Day dermal toxicity study in rabbits

TEST MATERIAL: MESUROL technical

PC. No.: 100501

MRID Number: 409223-01

Tox Chemical No.: 578B

SYNONYMS: Methiocarb; Metmarcapturon; Mercaptodimethur

STUDY NUMBER: 51901

SPONSOR: Mobay Corporation, Health Environment and Safety Corporate Toxicology, Stanley Research Center, Stilwell, Kansas 66085-9101

TESTING FACILITY: Bio-Research Laboratories, Ltd., Senneville, Quebec

TITLE OF REPORT: A 21-Day Dermal Toxicity Study of MESUROL® Technical in Albino Rabbits

AUTHORS: B.G. Procter

REPORT ISSUED: November 23, 1988

CONCLUSIONS: MESUROL technical was administered dermally to New Zealand white rabbits for 6 hours/day (±5%) for 21 days at doses of 0, 60, 150, and 375 mg/kg/day. No treatment-related effects were observed at either the low or mid doses. Although, at the highest dose administered (375 mg/kg/day), males showed statistically significantly decreased food consumption primarily during the second week of the study and statistically significantly decreased plasma cholinesterase activity on study day 21, the changes were of insufficient magnitude and duration to be considered adverse. No effect was observed in females at any dose. Five animals (1 low-dose male and 1 high-dose male; 2 low-dose females and 1 mid-dose female) had symptoms consistent with mucoid enteritis. Body weight, food consumption, and plasma cholinesterase data were analyzed both including and excluding these animals. Minor differences in the results were obtained if the ill animals were excluded, but the overall conclusions of the study were unaffected. Convincing LOELs for systemic

toxicity and cholinesterase inhibition cannot be established. A higher dose of the test material should have been administered. The NOEL for systemic toxicity and cholinesterase inhibition is ≥ 375 mg/kg/day.

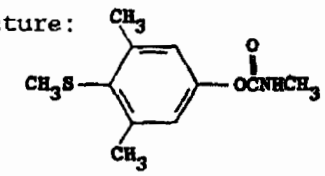
CORE CLASSIFICATION: This study is considered to be Core Supplementary since the test material was not administered at the limit dose for this type of study (1,000 mg/kg/day) or at a dose sufficient to elicit clear cut signs of toxicity. In addition, the study reported mucoid enteritis in 5 animals based on clinical symptoms observed in these animals; however individual animal data for clinical observations were not presented to verify this information. Althought the study cannot be upgraded, for purposes of completeness, the sponsor is requested to submit individual animal data for clinical observations, a signed statement certifying test material purity as well as a description of the methodology used for the cholinesterase analyses.

A. MATERIALS, METHODS, AND RESULTS

1. Test Article Description

Name: MESUROL® technical

Structure:



Batch number: 86I004

Purity: 99.3%

Physical property: White, crystalline

Stability: Reported by the sponsor to be stable under conditions of normal use

2. Test Substance Analyses for Purity and Stability

The purity of the test material was reported by the study author to be 99.3% (for Batch 86I004). However, a signed statement certifying the purity analysis was not provided. The sponsor reported the compound to be stable under conditions of normal use.

3. Animals

Male and female New Zealand albino rabbits were received from Maple Lane Farm, Clifford, Ontario. Animals were housed individually in stainless steel cages in a room with a 12-hour light/dark cycle. Temperature and relative humidity were maintained at 17±3°C and 30-70%, respectively. Tap water and Purina Rabbit Chow #5322 were provided *ad libitum*. Diets were supplemented with lettuce and

carrots. The rabbits were acclimated for 2 weeks prior to dosing. The rabbits were identified with individual ear markings. At the time of exposure, the animals were approximately 12 weeks of age; the body weights of males ranged from 2.4 to 3.0 kg and the body weights of females ranged from 2.5 to 3.3 kg.

The animals received a physical examination prior to treatment. Rabbits considered healthy were randomly assigned to four treatment groups (5/sex/group) with the aid of a computer-generated randomization procedure. Group assignments were as follows:

Dose Group	Dose Level (mg/kg/day)
Group 1 - Vehicle control[a]	0
Group 2 - Low-dose	60
Group 3 - Mid-dose	150
Group 4 - High-dose	375

[a]Physiologic saline (0.9%; 1 mL) was applied to the vehicle controls.

4. Test Procedure

Hair was removed (by shaving) from the back of each rabbit at least 24 hours prior to first application of the test material and thereafter when necessary. The shaved portion constituted an exposure area of approximately 10% of the total body surface. The appropriate amount of test material for each individual rabbit (adjusted according to the most recent body weight determination) was moistened with a small amount of physiologic saline (0.5 mL for group 2 and 1.0 mL for groups 3 and 4) and applied evenly to a piece of gauze (using a metal spatula). The treated gauze was inverted and placed on the treatment site of each rabbit. Gauze moistened with 1 mL of physiologic saline was placed on the control rabbits. The application site was occluded with impervious wrap during the exposure period. The animals were exposed to the test material or vehicle control for approximately 6 hours (±5%) per day for 21 consecutive days. On day 3, 2 mid-dose animals were exposed for only approximately 5.5 hours, but this probably did not affect the results of the study. At the end of the exposure period, the treated area of each animal was wiped with a gauze pad moistened with tap water. To prevent ingestion of any residual test material, the animals wore Elizabethan collars between exposures.

5. Statistical Methods

Food consumption, clinical pathology (hematology, clinical chemistry, red blood cell, plasma, and brain cholinesterase), and organ weight data were analyzed statistically. Bartlett's test was performed to determine if groups had homogenous variances. If the variances were homogenous, an ANOVA was used to determine significance. If there was a significant difference, Dunnett's test was used to determine which means were significantly different from controls. If variances were heterogeneous, the Kruskal-Wallis test and a summed rank test (Dunn) were used.

6. General Observations

(a) Mortality/moribundity/survival

Animals were observed for mortality/moribundity daily.

One low-dose female died on study day 10, and 1 low-dose male became moribund and was subsequently sacrificed on study day 12. The study author suggested that these deaths were due to mucoid enteritis. No other deaths occurred during the study.

(b) Clinical observations/dermal reactions

Observations for overt signs of toxicity were made twice daily throughout the period of exposure. Dermal irritation was evaluated by the Draize method immediately before daily test material application.

Individual animal data regarding clinical observations were not presented in the study report. However, the study report indicated that no dermal irritation (erythema or edema) was observed and that incidences of softening of the feces, fecal staining in the urogenital region, and nasal discharge noted occasionally during the study did not appear to be treatment related. The study report also noted that several animals (1 low-dose male, 2 low-dose females, 1 mid-dose female, and 1 high-dose male) displayed signs consistent with mucoid enteritis. These included moderate/severe staining of the urogenital area, yellow mucoid material adhering to the fur of the perineum, and moderate epistaxis. In addition to these signs, the low-dose female that died on study and the low-dose male that was sacrificed moribund showed decreased fecal output, emaciation, and reduced motor activity prior to death. These findings could not be confirmed by the reviewers because the individual data was not available.

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(c) Body weights/food consumption

Body weights--All animals were weighed immediately prior to initial treatment, twice a week during the exposure period, and immediately prior to sacrifice.

No statistically significant effects on body weight or body weight gain were observed irrespective of whether animals with mucoid enteritis were included or excluded from the analyses.

Food consumption--Food consumption measurements were taken prior to initiation (not provided in final report), and every other day during treatment.

Food consumption data are presented in Table 1. Data are presented both including and excluding the data for the 5 animals with mucoid enteritis. Statistically significant decreases in food consumption among high-dose males were observed irrespective of whether animals with mucoid enteritis were included or excluded from the data analyses. The unadjusted high-dose male data showed a significant decrease in food consumption on days 7-9 and on days 13-15, whereas the adjusted high-dose male data showed significant decreases in food consumption on days 7-9 and days 11-13. These intervals were the only periods in which food consumption differed significantly from controls.

7. Clinical Pathology

Samples for standard hematology and clinical chemistry analyses were collected 1 week before treatment began and at terminal sacrifice. Animals were fasted for 12 hours before blood was taken from the auricular artery. Because of a technical error, 15 animals/sex were not fasted prior to the collection of the sample during the pretreatment period. This error was not considered to have affected the results of the study.

In addition, plasma and red blood cell cholinesterase levels were determined in all animals at several additional intervals. Samples (1 mL whole blood per rabbit) were taken once during pretreatment, and immediately following the 6-hour exposure period on study days 1, 7, 14, and 21. In the high-dose groups, additional samples were collected 10 hours later (16 hours postexposure) to test for recovery of cholinesterase activity. Following sacrifice, the left half of the brain of each animal was frozen at -20°C and later analyzed for brain cholinesterase activity. All biochemical tests were performed via automated analysis using a Hitachi 705.

The parameters checked (X) below were evaluated

(a) Hematology

X Hematocrit (HCT)*
X Hemoglobin (HGB)*
X Leukocyte count (WBC)*
X Erythrocyte count (RBC)*
X Platelet count*
X Reticulocyte count (RETIC)
X Leukocyte differential count
X Mean corpuscular HGB (MCH)
X Mean corpuscular HGB concentration (MCHC)
X Mean corpuscular volume (MCV)
X Activated prothrombin time (PT)*
X Activated partial thromboplastin time (APTT)*

*Recommended by Subdivision F (November 1984) Guidelines

No changes were observed in any hematology parameter that were considered to be treatment related. Low-dose males had a significant increase in lymphocyte count when compared to controls (178%), but this effect did not increase with dose and was therefore not considered to be treatment related.

(b) Blood (clinical) chemistry

Electrolytes

X Calcium*
X Chloride*
Magnesium*
X Phosphorus*
X Potassium*
X Sodium*

Enzymes

X Alkaline phosphatase (ALP)
X Cholinesterase (plasma, RBC, brain)
Creatinine phosphokinase
X Lactic acid dehydrogenase
X Glutamic oxaloacetic transaminase (GOT)*
X Glutamic pyruvic transaminase (GPT)*
X Gamma glutamyltranspeptidase (GGT)

Other

X Albumin*
X Albumin/globulin ratio
X Blood creatinine*
X Blood urea nitrogen*
X Cholesterol*
X Globulins
X Glucose*
X Total bilirubin*
Direct bilirubin
X Total protein*
X Triglycerides

*Recommended by Subdivision F (November 1984) Guidelines

Table 2 summarizes data on plasma cholinesterase parameters. Data are presented both including and excluding rabbits with mucoid enteritis. Plasma cholinesterase values were slightly lower when compared to control in both the mid- and high-dose males, but statistical significance was achieved only in high-dose males. Significant decreases (~25-30%) in plasma cholinesterase activity in high-dose males were observed 6 hours postexposure on days 14 and 21 in the unadjusted data. When data

excluding animals with mucoid enteritis were analyzed, a statistically significant decrease (20%) was seen in plasma cholinesterase activity in high-dose males at day 21, 6 hours postexposure. Plasma cholinesterase activity was determined again 16 hours postexposure in high-dose animals, and activity was similar to the 6-hour postexposure measurement. There were no statistically significant changes in erythrocyte and brain cholinesterase activities relative to the appropriate controls. [Note: Some type of assay or calculation error appeared to have occurred during the measurement of the erythrocyte cholinesterase activity for both sexes on day 1, 6 hours post exposure because the reported values from all groups were much larger than the preceding or subsequent values.]

No other treatment-related changes were observed in the clinical chemistry analyses. Low-dose females showed a slight but statistically significant decrease in total bilirubin levels when compared to controls (5%), and mid-dose females showed a slight but significant increase in total bilirubin levels when compared to controls (5%). A significant increase was seen in cholesterol levels in the high-dose males (49%). These changes were not dose related and were not supported by histopathological evidence of organ toxicity; thus, they do not appear to be biologically significant.

8. Sacrifice and Pathology

All surviving animals were sacrificed at the end of the treatment period by an overdose of sodium pentobarbital administered intravenously, followed by exsanguination. Necropsies were performed on the male animal sacrificed moribund, the treated female found dead, and on all animals surviving to terminal sacrifice. Samples of the tissues listed below were taken from all animals and preserved in 10% neutral buffered formalin (eyes, testes, and optic nerves were preserved in Zenker's fluid). Tissues indicated by an "X" in the following list were taken from the control and high-dose animals, embedded in paraffin wax, sectioned, and examined microscopically. In addition, organs (from all animals) indicated by "XX" were weighed prior to fixation.

Digestive System	Cardiovascular/Hematologic	Neurologic
Tongue	Aorta	XX Brain[a]
Salivary glands	XX Heart	Peripheral nerve (sciatic nerve)
Esophagus	Bone marrow	Spinal cord (three levels)
Stomach	Lymph nodes	XX Pituitary
Duodenum	XX Spleen	Eyes (optic nerve)
Jejunum	Thymus	
Ileum		
Cecum	Urogenital	
Colon		
Rectum	XX Kidneys*	Glandular
XX Liver*	Urinary bladder	
Gallbladder	XX Testes	XX Adrenals
Pancreas	Epididymides	Lacrimal gland
	Ureters	Mammary gland
Respiratory	Seminal vesicle, prostrate	XX Thyroids*
	XX Ovaries	XX Parathyroids*
Trachea	Uterus, oviduct	Harderian glands
XX Lung*	Cervix, vagina	

Other

Bone (sternum and femur)
Skeletal muscle
Fat
X Skin (treated and untreated)*
X Any abnormalities*

*Recommended by Subdivision F (November 1984) Guidelines

[a]Right half was placed in fixative. Left half was used for cholinesterase analysis.

(a) Macroscopic

No treatment-related gross pathological changes were observed. Liver discoloration and lung depression were noted at similar incidences in all groups.

(b) Organ weights and organ-to-body-weight ratios

No treatment-related changes in organ weights were observed. Statistically significant decreases in absolute heart weights of low- and high-dose males (12% and 13%, respectively) and increases in absolute and organ-to-body-weight ratios of the left gonad were noted in the mid-dose males (31% and 23%, respectively). However, these values were not considered to be treatment related since the effects did not increase with dose.

(c) Microscopic

There were no microscopic findings in any of the tissues examined that could be attributed to treatment with the test compound. Histological evaluation of the treated skin revealed subtle changes consisting of various combinations of hyperkeratosis, epidermal hyperplasia, and mixed cell infiltration in the upper dermis. These changes were observed in both the control and high-dose animals with a greater incidence in the controls.

B. DISCUSSION

With the exception that a higher dose of test material should have been used (see below), no deficiencies were noted in the design or conduct of this study. The information presented in the study report was generally supported by the individual animal data, but no individual animal data were located to substantiate conclusions regarding clinical observations. This is particularly important in this study since it was concluded that a number of animals had mucoid enteritis based on symptoms observed during the study. Furthermore, two preterminal deaths were attributed to the mucoid enteritis.

Because of the possibility that animals suffering from mucoid enteritis may have confounded interpretation of the results of the study, select data were analyzed both including and excluding results from the affected animals. Irrespective of inclusion or exclusion of these rabbits, the only changes observed in this study were seen in the high-dose males. High-dose males had statistically significantly decreased food consumption (approximately 20-30% less than control) primarily during the second week of the study and statistically significantly decreased plasma cholinesterase activity (approximately 20% less than control) by the end of the study. However, these changes did not appear to be adverse and it was difficult to judge whether or not they were related to treatment. The plasma cholinesterase inhibition was not dramatic and no effects were observed on erythrocyte or brain cholinesterase activity. The decrease in food consumption was not accompanied by body weight changes or other signs of systemic toxicity and by the end of the study, high-dose male food consumption values approached or exceeded those of the control or 60 mg/kg/day dose group. There was no evidence of systemic toxicity or statistically significant cholinesterase activity inhibition in females at any dose level.

Convincing LOELs for systemic toxicity and cholinesterase inhibition cannot be established. A higher dose of the test material should have been administered. The NOEL for systemic toxicity and cholinesterase inhibition is $\geq$ 375 mg/kg/day.

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C. QUALITY ASSURANCE

A signed Good Laboratory Compliance Statement, a signed Quality Assurance Statement, and a list of Quality Assurance Inspections were included in the report.

Table 1. Mean (± SD) Food Consumption (g/animal) in Rabbits Exposed Dermally to MESUROL® Technical for 21 Days[a,b]

	Males -- Dose Group (mg/kg/day)					
	0	60		150	375	
Interval	Unadjusted[b]	Unadjusted[c]	Adjusted[d]	Unadjusted[b]	Unadjusted[b]	Adjusted[d]
-1-1	200.0±82.4	311.0±100.6	311.0±100.6	254.4±80.3	257.6±64.0	271.8±64.3
1-3	291.0±88.7	376.6±94.0	345.0±71.6	361.8±37.3	329.2±57.5	340.5±59.7
3-5	316.6±93.8	220.6±282.8	239.5±152.8	256.8±94.8	270.6±76.1	263.3±85.7
5-7	350.0±76.2	282.8±174.7	349.8±104.0	286.0±147.7	324.0±44.0	332.3±46.1
7-9	379.2±64.9	338.5±96.2	338.5±96.2	373.6±43.4	255.8±52.6*	258.0±60.4*
9-11	374.0±66.4	308.2±156.5	377.5±25.6	381.2±58.2	248.0±128.9	292.5±94.6
11-13	354.2±50.2	327.0±179.1	405.8±37.6	380.0±40.3	227.4±102.7	272.0±28.3*
13-15	363.4±62.8	381.5±47.8	381.5±47.8	382.6±32.6	256.6±88.9*	294.8±28.9
15-17	342.4±59.3	371.0±68.1	371.0±68.1	395.2±48.9	318.8±32.9	322.5±36.8
17-19	326.2±68.4	313.3±74.7	313.3±74.7	364.8±72.3	347.2±46.1	338.8±48.5
19-21	373.2±58.4	298.0±195.0	298.0±195.0	347.4±76.8	348.4±78.7	328.8±75.5

	Females -- Dose Group (mg/kg/day)					
	0	60		150		375
Interval	Unadjusted[b]	Unadjusted[e]	Adjusted[f]	Unadjusted[b]	Adjusted[d]	Unadjusted[b]
-1-1	327.8±156.1	247.4±145.0	243.0±146.7	294.6±125.2	318.0±130.5	251.0±99.3
1-3	348.4±79.7	382.0±84.4	399.0±93.6	331.4±74.8	327.0±85.6	383.4±64.3
3-5	289.0±123.8	286.8±106.4	305.3±99.4	245.4±137.7	287.5±116.1	274.0±125.2
5-7	338.2±167.5	311.0±178.5	388.0±101.8	257.0±188.3	320.5±142.8	359.8±125.4
7-9	382.6±30.1	319.2±181.9	392.7±84.3	295.8±179.7	368.0±91.0	384.4±77.3
9-11	361.8±32.7	402.0±32.8	405.7±39.1	311.6±161.8	381.8±45.7	394.2±47.1
11-13	353.0±95.6	374.3±47.8	379.0±57.4	372.2±94.1	400.3±80.9	443.0±166.0
13-15	382.6±110.2	420.8±56.5	428.0±66.8	372.8±60.5	378.0±68.5	375.8±61.3
15-17	413.6±103.9	367.3±31.3	362.0±36.2	400.0±81.6	381.3±80.9	385.0±45.2
17-19	407.0±122.3	372.3±40.9	372.3±40.9	377.0±107.7	351.5±105.6	309.8±63.5
19-21	442.4±114.7	376.8±50.7	389.7±53.4	385.2±92.8	367.8±97.3	353.6±38.5

[a]Source: Study No. 51901, Appendices No. 10-18.
[b]N=5
[c]N=5 until day 12; after day 12, N=4.
[d]N=4; animals with mucoid enteritis were excluded; data calculated by reviewers.
[e]N=5 until day 10; after day 10, N=4.
[f]N=3; animals with mucoid enteritis were excluded; data calculated by reviewers.

*Significant at $p<0.05$. Unadjusted data analyzed by Dunnett's test. Adjusted data analyzed by ANOVA with Scheffe's ad hoc test using the ABSTAT computer program.

Table 2. Mean (± SD) Plasma Cholinesterase Activity (U/L) in Rabbits Exposed Dermally to MESUROL® Technical for 21 Days[a]

	Males -- Dose Group (mg/kg/day)					
	0	60		150	375	
Interval	Unadjusted[b]	Unadjusted[c]	Adjusted[d]	Unadjusted[b]	Unadjusted[b]	Adjusted[d]
Pretest	363.0±43.09	369.4±58.11	361.5±63.93	331.4±83.45	336.8±63.10	340.3±91.31
Day 1						
6-hr	330.0±94.08	360.0±66.34	356.3±75.99	289.3±38.16	305.0±81.62	323.8±80.86
16-hr	ND	ND	ND	ND	303.8±40.60	298.3±44.63
Day 7						
6-hr	320.2±23.27	305.0±77.38	332.3±55.10	275.4±71.52	250.0±72.19	272.8±59.15
16-hr	ND	ND	ND	ND	258.0±75.95	277.3±72.26
Day 14						
6-hr	345.6±55.34	345.3±59.51	345.3±59.51	260.8±33.78	239.0±83.39*	266.5±65.04
16-hr	ND	ND	ND	ND	281.2±79.40	303.5±71.35
Day 21						
6-hr	360.6±34.11	339.3±47.17	339.3±47.17	303.6±31.33	274.4±44.41*	289.5±33.31*
16-hr	ND	ND	ND	ND	287.8±54.6	299.0±56.02

	Females -- Dose Group (mg/kg/day)					
	0	60		150		375
Interval	Unadjusted[b]	Unadjusted[e,*]	Adjusted[f]	Unadjusted[b]	Adjusted[d]	Unadjusted[b]
Pretest	371.8±70.70	409.2±78.95	422.3±104.64	314.4±66.15	323.8±72.47	347.0±54.87
Day 1						
6-hr	322.0±54.97	348.6±50.91	379.7±39.25	264.2±40.63	267.0±46.35	323.4±96.48
16-hr	ND	ND	ND	ND	ND	353.8±89.48
Day 7						
6-hr	336.4±34.70	302.2±71.00	334.3±67.31	242.4±86.04	271.0±66.46	281.2±82.93
16-hr	ND	ND	ND	ND	ND	309.0±83.41
Day 14						
6-hr	317.0±43.52	329.5±38.28	345.0±27.51	266.8±57.64	272.3±65.06	289.4±75.45
16-hr	ND	ND	ND	ND	ND	301.2±64.53
Day 21						
6-hr	340.8±39.04	345.0±46.83	362.7±37.63	293.8±63.90	294.0±73.79	317.2±44.21
16-hr	ND	ND	ND	ND	ND	307.6±30.97

[a]Source: Study No. 51901, Appendices No. 30-31.
[b]N=5
[c]N=5 until day 12; after day 12, N=4.
[d]N=4; animals with mucoid enteritis were excluded; data calculated by reviewers.
[e]N=5 until day 10; after day 10, N=4.
[f]N=3; animals with mucoid enteritis were excluded; data calculated by reviewers.

*Significant at $p<0.05$. Unadjusted data analyzed by Dunnett's test. Adjusted data analyzed by ANOVA with Scheffe's ad hoc test using the ABSTAT computer program.

ND = not determined

FINAL

010314

DATA EVALUATION REPORT

MESUROL® 75% Concentrate

Study Type: Acute Inhalation Toxicity Study in Rats

Study Title: Acute 4-Hour Inhalation Toxicity Study with MESUROL® 75% Concentrate in Rats

Prepared for:

Health Effects Division
Office of Pesticide Programs
Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by:

Clement International Corporation
9300 Lee Highway
Fairfax, VA 22031-1207

Date: February 12, 1993

Principal Reviewer:	Jay Meeks, B.S.	4/17/93 Date
Independent Reviewer:	John Liccione, Ph.D.	2/12/93 Date
QA/QC Manager:	Sharon Segal, Ph.D.	2/17/93 Date

Contract Number: 68D10075
Work Assignment Number: 2-08
Clement Number: 2-08-17
Project Officer: James E. Scott

EPA Reviewer: John Redden, M.S.
Review Section III, Toxicology Branch I/HED

Signature

3/10/93
Date

Acting EPA Section Head: Karen Hamernick, Ph.D.
Review Section III, Toxicology Branch I/HED

Signature

5/6/93
Date

DATA EVALUATION REPORT

STUDY TYPE: Guideline Series 81-3: Acute inhalation toxicity study in rats

EPA IDENTIFICATION NUMBERS

Tox. Chem. Number: 578B
MRID Number: 405181-03C
P.C. Number: 100501

TEST MATERIAL: MESUROL® 75% Concentrate

SPONSOR: Mobay Corporation, Agricultural Chemicals Division, Kansas City, MO

STUDY NUMBER: 86-041-18 ; Report No. 95623 ; Tox Report 984

TESTING FACILITY: Mobay Corporation, Stilwell, Kansas

TITLE OF REPORT: Acute 4-Hour Inhalation Toxicity Study with MESUROL® 75% Concentrate in Rats

AUTHOR: Shiotsuka, R.N.

STUDY COMPLETED: February 5, 1988

CONCLUSIONS: Estimated acute inhalation LC_{50}'s for MESUROL® 75% Concentrate with 95% confidence intervals following a 4-hour exposure:

0.479 mg/L for males
0.403 mg/L for females
MMAD 3.4 to 4.4 µm.

The NOEL for a 4-hour exposure:

<0.348 mg/L for male Sprague-Dawley rats based on compound-related decreases in weight gain and the occurrence of clinical signs of toxicity.

<0.234 mg/L for female Sprague-Dawley rats based on compound-related clinical signs and gross effects.

CORE CLASSIFICATION: ACCEPTABLE. This study satisfies the requirements according to Guideline Series 81-3 for an acute inhalation study.

TOXICITY CATEGORY: II--Warning

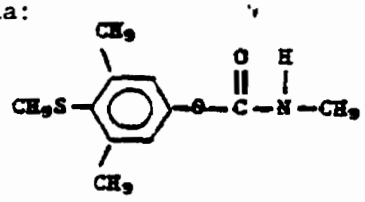
A. MATERIALS

1. Test Material

Compound: MESUROL® 75% Concentrate
Composition:

MESUROL technical	75.0%
Zeolex 7A	5.0%
Talcron MP 50-26	20.0%
	100.0%

Chemical name: 3,5-Dimethyl-4-(methylthio) phenol methylcarbamate
Common name: Methiocarb
CAS Registry number: 2032-65-7
Purity of material: 77.1%
Physical description: Off-white solid
Receipt date: Not reported
Batch number: 7035135
Storage conditions: The test material was stored frozen at -23°C.
Stability: The test material is expected to be stable under the intended conditions of storage and use during the study.
Structural formula:



2. Dose Levels:

Test Group

Material: MESUROL® 75% technical

Nominal Concentration (mg/L)	Mean Gravimetric Concentration (mg/L)
1.165	0.234
1.671	0.348
2.515	0.433
3.241	0.529

Controls

Material: Conditioned room air

3. Test Animals

Species: Rats
Strain: Sprague-Dawley; Sas: CD (SD)BR
Source: Sasco, Inc., Omaha, Nebraska
Receipt date: Not reported
Sex: Male and female
Number: 24 Males, 24 females; 6 males and 6 females were used for the control, 0.234, and 0.529 mg/L groups, whereas, 6 females were dosed at the 0.234 mg/L dosage level and 6 males were dosed at the 0.433 mg/L dosage level
Housing: Individually
Age at exposure: 8-11 Weeks
Weight: 216-278 g (males), 190-234 g (females)
Feed: Purina® Laboratory Rodent Chow #5001 and water ad libitum, except during the exposure period
Assignment: Animals were assigned to groups using a computer-generated randomization procedure

B. TEST PERFORMANCE

Inhalation Chamber

Animals (10/group) were exposed head-only in a 60-liter cylindrical chamber. The animals were positioned in holding tubes (attached near the middle portion of the inhalation chamber) to ensure that the head was exposed to the test atmosphere and the body remained outside of the chamber. Prior to exposure, the chamber was equilibrated by generating the test atmosphere for at least 30 minutes. The rats were exposed to test or control (room air) atmospheres for 4 hours.

Dose Preparation/Generation of Test Atmosphere

The test material was generated as dust using a Wright Dust Feeder. Prior to exposure, the test material was ground into fine powder using a mortar and pestle. The test material was compressed into a dust feed cup at 186 psi. The flow of dry, filtered air through the generator was 20 liters/minute. The test material, generated by the dust feeder, was mixed with respirable air in the upper portion of the exposure chamber. The airflow of the exposure chamber, monitored continuously by a rotameter, was recorded approximately every half hour.

Analytical Determinations

At least four gravimetric determinations (0.5, 1.5, 2.5-2.6, and 3.5 hours of exposure) of the chamber atmosphere concentrations were conducted at each dose volume. Additional samples were made for exposure concentrations of 0.234, 0.433, and 0.529 mg/L. Samples were drawn through

DM-800 Metricel membrane filters (0.8 µm) from the animals' breathing zone at a rate of 3.5-3.9 Lpm for 1.01-1.60 minutes and the amount of test material on the filter paper was determined gravimetrically using a Cahn-27 Automatic Electrobalance. The gravimetric concentration was determined by dividing the mass of the test substance collected on the filter paper by the volume of air sampled.

The nominal concentration of the test material was calculated by dividing the weight loss of the test substance by the total airflow passing through the chamber.

Results of both the gravimetric and nominal concentration analyses are presented below:

Nominal Concentration (mg/L)	Mean Gravimetric Concentration (mg/L)
1.165	0.234
1.671	0.348
2.515	0.433
3.241	0.529

The nominal concentration at each dose level tested was higher (79-84%) than the corresponding gravimetric concentration, perhaps reflecting losses of the aerosol in the chamber.

Chamber Monitoring

Chamber temperature and chamber relative humidity were recorded continuously during the exposure period. The chamber temperature ranged from 18.7°C to 23.5°C for the test groups and from 17.5°C to 18.3°C for the control group. The relative humidity ranged from 20 to 41% for the test groups and 44-46% for the control group. The relative humidity was lower in the test group chamber in comparison to the control group chamber because test animals were exposed to dry filtered air supplied through the dust feeder and the control animals were exposed to conditioned room air.

Particle-Size Determinations

A seven-stage Mercer Cascade Impactor was used to determine the particle-size distribution of the aerosol in the exposure chamber. At least two samples of the aerosol (at each dose level) were collected near the breathing zone of the rats for analyses. Mass median aerodynamic diameters ranged from 3.4 µm to 4.4 µm and the mean geometric standard deviations ranged from 2.2 to 9.2. Data on particle-size determinations were insufficient to assess the percentage of particles within the respirable range.

Body Weights/Clinical Observations/Gross Necropsies

Animals were weighed prior to treatment on day 0 and on days 3, 7, and 14 (just prior to sacrifice) or at death. The animals were observed for mortality on the day of exposure and for signs of toxicity at approx-

imately 0.5 hours postexposure and just prior to the end of the normal work day. Detailed physical observation data were recorded twice daily (once on weekends) during the 14-day post-observation period. Gross postmortem examinations were performed on all animals that died during the study as well as those animals that survived until the end of the 14-day postexposure period.

Statistics

The LC_{50} and 95% confidence limits were calculated by the probit method.

C. RESULTS AND STUDY AUTHOR'S CONCLUSIONS

Mortality

Inhalation exposure to concentrations of 0.234, 0.348, 0.433, and 0.529 mg/L resulted in mortality rates of 0, 17, 17, and 83%, respectively. No deaths occurred in the control groups. Deaths (as indicated by the mortality rate) were treatment related with the majority occurring in the high-dose group. Most deaths occurred between days 0 and 2. One female rat dosed at 0.348 mg/L died on Day 5. Based on these findings, the LC_{50} values and 95% confidence limits were calculated as follows:

Sex	LC_{50} (mg/L)*	95% Confidence Limits (mg/L)*
Males	0.479	0.415-0.561
Females	0.403	0.304-0.562

*mg/L of MESUROL® 75% Concentrate

MESUROL®75% Concentrate Toxicity

Compound-related clinical observations noted in most of the animals exposed to 0.234, 0.433, or 0.529 mg/L included oral and/or nasal discharge, salivation, hypoactivity, and tremors. Ocular opacity was observed in 1 animal dosed at 0.234 mg/L on day 1 and persisted until study termination (day 14). Other clinical observations noted were sporadic. Most of the clinical observations noted were seen as early as day 0 and persisted until Day 7.

Body Weights

Treatment-related body weight losses were observed in males and females on day 3 postexposure. Surviving animals dosed at 0.234, 0.348, 0.433, and 0.529 mg/L lost approximately 10.8, 7.0, 10.2, and 11.5% of their body weight, respectively, on day 3 when compared to their initial body weight (day 0). Most of the control animals gained weight during the study. Body weight recovery occurred during the study, and body weights of most

of the surviving animals were in excess of their pre-exposure body weights by termination of the study.

Gross Necropsy

Treatment-related gross observations noted in the animals found dead at doses of 0.433 and/or 0.529 mg/L included red turbinates, lacrimation, salivation/oral staining, and nasal staining/discharge. Ocular irritation noted in all treatment groups included ocular opacity, rough corneas and/or neovascularization. Ocular lesions were present in 1 low-dose female and 2 mid-dose males sacrificed at study termination. No ocular lesions were noted in the control animals.

D. REVIEWERS' COMMENTS

This study is classified as Core Acceptable.

E. QUALITY ASSURANCE MEASURE

A signed Quality Assurance Statement dated February 5, 1988 was presented. A Good Laboratory Practice compliance statement was included.

FINAL

010314

DATA EVALUATION REPORT

MESUROL

Study Type: Mutagenicity: Sister Chromatid Exchange Assay in Chinese Hamster Ovary (CHO) Cells

Prepared for:

Health Effects Division
Office of Pesticide Programs
Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by:

Clement International Corporation
9300 Lee Highway
Fairfax, VA 22031-1207

Principal Reviewer Nancy E. McCarroll, B.S Date 12/17/92

Independent Reviewer Lynne Haber, Ph.D. Date 12/17/92

QA/QC Manager Sharon Segal, Ph.D. Date 12/17/92

Contract Number: 68D10075
Work Assignment Number: 2-08
Clement Number: 14
Project Officer: James Scott

GUIDELINE § 84: MUTAGENICITY
IN VITRO SCE

MUTAGENICITY STUDIES

EPA Reviewer and Acting Section Head: Karen Hamernik, Ph.D.
Review Section III,
Toxicology Branch I/HED (H-7509C)

Signature: [signature] 12/24/92
Date: [signature] 5/6/93

DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity: Sister chromatid exchange assay in Chinese hamster ovary (CHO) cells

EPA IDENTIFICATION Numbers:

PC Code: 100501

Tox Chem. Number: 578B

MRID Number: 405081-02

TEST MATERIAL: Mesurol® technical

SYNONYMS/CAS NUMBER: 3,5-Dimethyl-4-(methylthio)phenol methylcarbamate; methiocarb; metmarcapturon; mercaptodimethur/2032-65-7

SPONSOR: Mobay Chemical Corp., Stilwell, KS

STUDY NUMBER: Study Report Number: 91334; Laboratory Study Number: T4522.334; Toxicology Report Number; 790

TESTING FACILITY: Microbiological Associates, Inc., Bethesda, MD

TITLE OF REPORT: Sister Chromatid Exchange Assay in Chinese Hamster Ovary (CHO) Cells Test Article Mesurol® Technical

AUTHOR: D.L. Putman

REPORT ISSUED: September 25, 1986

CONCLUSIONS--EXECUTIVE SUMMARY: Mesurol technical was investigated for the potential to induce sister chromatid exchange (SCE) in Chinese hamster ovary (CHO) cells. Owing to severe cytotoxicity and cell-cycle delay, cultures exposed to nonactivated doses of 2-40 µg/mL and S9-activated doses of 4-40 µg/mL were harvested 26-32 hours posttreatment. Results indicated that the test material was cytotoxic at ≥10 µg/mL -S9 and at 40 µg/mL +S9 but not genotoxic. We conclude, therefore, that mesurol technical was adequately tested and found to be nongenotoxic in a well-controlled assay.

STUDY CLASSIFICATION: Acceptable. The study satisfies Guideline requirements (§84-4 for genetic effects Category III, Other Mutagenic Mechanisms.

A. MATERIALS:

1. Test Material: Mesurol technical

Description: White crystalline powder
Identification numbers: Batch number: 0030058, Formula number 605250
Purity: 97.7%
Receipt date: December 27, 1985
Stability: Not reported
Contaminants: None listed
Solvent used: Dimethyl sulfoxide (DMSO)
Other provided information: The test material was stored with a desiccant at room temperature, protected from light. The frequency of test material solution preparation was not reported and analytical determinations of the dosing solutions were not performed.

2. Control Materials:

Negative: Ham's F-12 or McCoy's 5A medium supplemented with 10% fetal calf serum (FBS), 2 mM glutamine, and antibiotics.

Solvent/final concentration: DMSO/1%

Positive:

Nonactivation (concentrations, solvent): Triethylenemelamine (TEM) was prepared in distilled water to yield a final concentration of 0.025 µg/mL.

Activation: (Concentration, solvent): Cyclophosphamide (CP) was prepared in distilled water to yield a final concentration of 2.5 µg/mL.

3. Activation: S9 derived from adult, male Sprague-Dawley

x Aroclor 1254	x induced	x rat	x liver
____ phenobarbital	____ noninduced	____ mouse	____ lung
____ none		____ hamster	____ other
____ other		____ other	

The S9 fraction was prepared by the performing laboratory and was characterized for the ability to convert 2-aminoanthracene and 7,12-dimethylbenzanthrancene to mutagenic forms in Salmonella typhimurium TA100.

The composition of the S9 mix per mL of growth medium containing 2.5% (FBS) was:

NADP	1.4 mg
Isocitric acid	2.7 mg
S9 homogenate	15 µL

4. Test Compound Concentrations Used:

   (a) Preliminary cytotoxicity assay: Nine doses (0.1, 0.3, 1, 3, 10, 30, 100, 300, and 1000 µg/mL) were evaluated in the presence and absence of S9 activation.

   (b) SCE assays:

   - Nonactivated conditions: 2, 4, 10, 20, and 40 µg/mL.
     Note: Owing to an unspecified procedural error, the initial assay was aborted; presented results are from a repeat trial.
   - S9-activated conditions: 4, 10, 20, and 40 µg/mL

5. Test Cells: Chinese hamster ovary (CHO) cells, CCL61, were obtained from the American Type Culture Collection, Rockville, MD. CHO cells were grown for 16-24 hours prior to use.

   Properly maintained? Yes.
   Cell line or strain periodically checked for mycoplasma contamination? Not reported.
   Cell line or strain periodically checked for karyotype stability? Not reported.

B. TEST PERFORMANCE:

1. Cell Treatments:

   Cells exposed to the test compound, solvent, or positive controls for: 26-32 hours (nonactivated) 2 hours (activated)

2. Preliminary Assay: Prepared cultures, seeded at $5x10^5$ cells/flask, were exposed with or without S9 activation to half-log dilutions of the test material (0.1 to 1000 µg/mL) or the solvent control (DMSO).

   In the nonactivated system, cells were treated with the test material for 4 hours; with S9 activation, the treatment lasted for 2 hours. Following the specified exposure time, cells were washed, refed complete medium containing 0.01 mM BrdU and reincubated for 20-24 hours. Colcemid (final concentration, 0.1 µg/mL) was present for the last 2 hours of incubation.

   Cells were trypsinized and counted to determine relative cell growth. Metaphases were harvested, fixed and stained using the modified fluorescent-plus-Giemsa technique of Perry and Wolff.[1] An unspecified number of cells from each dose group were examined for the percentage of first (M_1), second (M_2), and third (M_3) division metaphases. Based on these results, dose selection and harvest times were established for the SCE assay.

[1]Perry, P., and Wolff, S. (1974). New Giemsa method for the differential staining of sister chromatids. Nature. 251:156-158.

3. SCE Assay:

(a) Treatment: Prepared cultures (in duplicate), seeded at $5x10^5$ cells/flask, were exposed to the selected test material doses, the negative control (culture medium), the solvent control (DMSO), or the positive controls (TEM -S9 or CP +S9) in a manner similar to that described for the preliminary cytotoxicity assay, with the following exceptions:

- In the nonactivated assay, cells were dosed for 26-32 hours. BrdU (0.01 mM) was added after the first 2 hours of incubation.
- Following the 2-hour S9-activated exposure, cells were reincubated in the presence of 0.01 mM BrdU for 24-30 hours.

(b) Slide analysis: All slides were coded prior to scoring. An unspecified number of metaphase cells per group were scored to determine the percentage of first (M_1), second (M_2), or third (M_3) division metaphases. Fifty M_2 cells per group (25/culture) were scored for the frequency of SCEs.

(c) Statistical methods: The data were evaluated for statistical significance at p values of 0.05 and 0.01 by the Student's t-test.

(d) Evaluation criteria:

(1) Assay validity: The assay was considered valid if the mean number of SCEs/cell in the negative control did not exceed 16 and the mean number of SCEs/cell in the positive control group was ≥2-fold higher than the negative control group.

(2) Positive response: The test material was considered positive if it caused a dose-related ≥2-fold increase in the mean number of SCEs/cell over a minimum of three dose levels. In the absence of a doubling over background, the results were considered positive if a dose-dependent and significant increase in the SCE frequency was obtained at ≥3 doses.

C. REPORTED RESULTS:

1. Preliminary Cytotoxicity Assay: Severe cytotoxicity, as indicated by the marked reduction in cell survival and/or absence of mitotic cells was observed at the three highest nonactivated levels (100, 300, and 1000 µg/mL) and at S9-activated doses ≥300 µg/mL. Cell-cycle delay was also apparent at 10 and 30 µg/mL -S9 and ≥30 µg/mL +S9 (Table 1). No cytotoxicity or clear evidence of interference with progression through the cell cycle was evident at lower doses

TABLE 1. Representative Results from the Preliminary Cytotoxicity Assay with Mesurol Technical

Substance	Dose	S9 Activation	% Cells[a] M_1	% Cells[a] M_2	Relative Percent Growth
Solvent Control					
Dimethyl sulfoxide	1%	-	7	93	100
	1%	+	2	98	100
Test Material					
Mesurol technical	3 µg/mL[b]	-	13	87	97
	10 µg/mL	-	49	51	86
	30 µg/mL[c]	-	68	32	59
	3 µg/mL[b]	+	5	95	107
	10 µg/mL	+	14	86	84
	30 µg/mL	+	55	45	66
	100 µg/mL[c]	+	100	0	35

[a]Percent cells in the first (M_1) or second (M_2) division; no M_3 cells were scored.
[b]Lower doses (0.1, 0.3, and 1.0 µg/mL (+/-S9) had no appreciable cytotoxic effects.
[c]Higher levels (100, 300, and 1000 µg/mL -S9 and 300 and 1000 µg/mL +S9) were severely cytotoxic and no metaphase cells were recovered.

Note: Data were extracted from the study report; see CBI pp. 13 and 14.

(≤3 µg/mL -S9; ≤10 µg/mL +S9). Based on these data, 2-40 µg/mL -S9 and 4-40 µg/mL +S9 were evaluated in the SCE assay; cells were harvested 26-32 hours posttreatment in both the nonactivated and S9-activated phase of testing.

2. SCE Assay: Representative findings from the nonactivated and S9-activated SCE assay conducted with mesurol technical are presented in Table 2. Nonactivated data were from a repeat test; the initial trial was terminated because of an unspecified procedural error. Few, if any, mitotic cells were recovered from cultures treated with the two highest nonactivated doses of mesurol (20 and 40 µg/mL). Cell-cycle delay was also noted in cultures treated with 10 µg/mL -S9; cytotoxicity was not apparent at lower levels. There was also no dose-related genotoxic effect. Our reviewers agree with the study author that the statistically significant ($p<0.05$) increase in mean SCEs/cell at the lowest assayed nonactivated dose (2 µg/mL) was not biologically meaningful since there was no dose-related response. Under S9-activated conditions, slight cell-cycle delay was seen at the high dose (40 µg/mL). There were, however, no significant increases in the SCE frequency at any dose or cytotoxic response at lower concentrations. By contrast, significant ($p<0.01$) genotoxicity was obtained in cultures exposed to the nonactivated (0.025 µg/mL TEM) and S9-activated (2.5 µg/mL) positive controls.

From the overall findings, the study author concluded that mesurol technical was negative both with and without S9 activation in this test system.

D. REVIEWERS' DISCUSSION/CONCLUSIONS: We assess that the study was properly conducted and that the study author interpreted the data correctly. Under nonactivated and S9-activated conditions, mesurol technical was tested to cytotoxic levels (≥10 µg/mL -S9; 40 µg/mL +S9) but failed to induce a genotoxic response in CHO cells. Additionally, the sensitivity of the test system to detect SCE activity was adequately demonstrated by the significant ($p<0.01$) increase in SCEs/cell obtained in cultures exposed to 0.025 µg/mL TEM-S9 or 2.5 µg/mL CP +S9. We conclude, therefore, that the study provided acceptable evidence that mesurol technical was not genotoxic in this cultured mammalian cell test system.

E. QUALITY ASSURANCE MEASURES: Was the test performed under GLPs? Yes. (A signed Quality Assurance Statement dated September 23, 1986, was present.)

F. CBI APPENDICES: Appendix A, Materials and Methods, CBI pp. 8-11; Appendix B, Protocol, CBI pp. 19-24.

CORE CLASSIFICATION: Acceptable. The study satisfies Guideline requirements (§84-4) for genetic effects Category III, Other Mutagenic Mechanisms.

TABLE 2. Representative Results of the Sister Chromatid Exchange (SCE) Assay in Chinese Hamster Ovary (CHO) Cells Treated with Mesurol Technical

Substance	Dose	S9 Activation	Number of Metaphases Scored	% Cells[a] M_1	% Cells[a] M_2	% Cells[a] M_3	Average SCEs/ Chromosome[b]	Group Mean SCEs/Cell ±S.D.
Negative Control								
Culture medium	--	-	50	4.5	89.5	6.0	0.61	11.48±4.60
	--	+	50	6.5	92.5	1.0	0.48	9.13±3.44
Solvent Control								
Dimethyl sulfoxide	1%	-	50	8.0	89.0	3.0	0.59	11.20±3.92
	1%	+	50	6.5	90.5	2.5	0.50	9.60±2.65
Positive Control								
Triethylene-melamine	0.025 µg/mL	-	32[c]	27.5	70.0	2.5	1.74	35.16±11.97**
Cyclophosphamide	2.5 µg/mL	+	50	7.5	91.5	1.0	1.31	25.06±7.00**
Test Material								
Mesurol technical	2 µg/mL	-	50	8.0	86.0	6.0	0.68	12.98±4.51*
	4 µg/mL	-	50	8.5	89.5	2.0	0.61	11.62±3.77
	10 µg/mL[d]	-	50	37.5	62.5	0.0	0.60	11.54±4.29
	20 µg/mL[e]	+	50	11.0	88.0	1.0	0.53	10.07±3.42
	40 µg/mL	+	50	20.0	77.0	3.0	0.36	6.90±3.13

[a]Average percent cells in first (M_1), second (M_2), or third (M_3) division; calculated by our reviewers
[b]Average value from duplicate cultures; calculated by our reviewers
[c]Only 7 M_2 cells in one of the two replicate cultures were scored.
[d]Higher levels (20 and 40 µg/mL) were severely cytotoxic.
[e]Results for lower doses (4 and 10 µg/mL) did not suggest a genotoxic response.

*Significantly higher ($p \leq 0.05$) than the solvent control by Student's t-test.
**Significantly higher ($p \leq 0.01$) than the solvent control by Student's t-test.

Note: Data were extracted from the study report; see CBI pp. 15-16

HED Records Center Series 361 Science Reviews - File 100501_0013000_060993_TX010314_R031539 - Page 41 of 104

010314

APPENDIX A

MATERIALS AND METHODS
CBI pp. 8-11

METHIOCARB
6-9-1993 TXR 010314

Page _____ is not included in this copy.

Pages 43 through 46 are not included in this copy.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

X FIFRA registration data.

_____ The document is a duplicate of page(s) _____.

_____ The document is not responsive to the request.

_____ Internal deliberative information.

_____ Attorney-client communication.

_____ Claimed confidential by submitter upon submission to the Agency.

_____ Personal privacy Information

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

APPENDIX B

Protocol

CBI pp. 19-24

010314

APPENDIX A

MATERIALS AND METHODS
CBI pp. 8-11

METHIOCARB
6-9-1993 TXR 010314

Page _____ is not included in this copy.

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_____ Identity of product inert ingredients.

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_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

X FIFRA registration data.

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_____ Internal deliberative information.

_____ Attorney-client communication.

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APPENDIX B

PROTOCOL AND PROTOCOL AMENDMENT
CBI pp. 20-29

METHIOCARB
6-9-1993 TXR 010314

Page _____ is not included in this copy.

Pages 54 through 63 are not included in this copy.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

X FIFRA registration data.

_____ The document is a duplicate of page(s) _____.

_____ The document is not responsive to the request.

_____ Internal deliberative information.

_____ Attorney-client communication.

_____ Claimed confidential by submitter upon submission to the Agency.

_____ Personal privacy Information

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

FINAL

010314

DATA EVALUATION REPORT

MESUROL® Technical

Study Type: Acute Inhalation Toxicity Study in Rats

Study Title: Acute Four-Hour Inhalation Toxicity Study with MESUROL® Technical in Rats

Prepared for:

Health Effects Division
Office of Pesticide Programs
Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by:

Clement International Corporation
9300 Lee Highway
Fairfax, VA 22031-1207

Date: February 12, 1993

Principal Reviewer: Jay Meeks 2/17/93
Jay Meeks, B.S. Date

Independent Reviewer: John Liccione 2/12/92
John Liccione, Ph.D. Date

QA/QC Manager: Sharon Segal 2/17/93
Sharon Segal, Ph.D. Date

Contract Number: 68D10075
Work Assignment Number: 2-08
Clement Number: 2-08-18
Project Officer: James E. Scott

EPA Reviewer: John Redden, M.S.
Review Section III, Toxicology Branch I/HED

Signature
3/10/93
Date

Acting EPA Section Head: Karen Hamernick
Review Section III, Toxicology Branch I/HED

Signature
5/6/93
Date

DATA EVALUATION REPORT

STUDY TYPE: Guideline Series 81-3: Acute inhalation toxicity study in rats

EPA IDENTIFICATION NUMBERS

Tox. Chem. Number: 578B
MRID Number: 404042-01
P.C. Number: 100501

TEST MATERIAL: MESUROL® technical (98.8% MESUROL®, active ingredient, a.i.)

SPONSOR: Mobay Corporation, Agricultural Chemicals Division, Kansas City, MO

STUDY NUMBER: 86-041-25 ; Report No. 94635 ; Tox Report No. 870

TESTING FACILITY: Mobay Corporation, Stilwell, Kansas

TITLE OF REPORT: Acute Four-Hour Inhalation Toxicity Study with MESUROL® Technical in Rats

AUTHOR: Shiotsuka, R.N.

STUDY COMPLETED: June 18, 1987

CONCLUSIONS: Estimated acute inhalation LC_{50}'s for MESUROL® technical with 95% confidence intervals following a 4-hour exposure:

0.585 (0.345-0.698) mg/L for males
0.433 (0.286-0.585) mg/L for females

The NOEL for a 4-hour exposure:

<0.199 mg/L for male and female Sprague-Dawley rats due to treatment-related clinical observations noted in animals exposed to 0.199 mg/L

MMAD 4.0 to 5.0 µm. (see note on page 4).

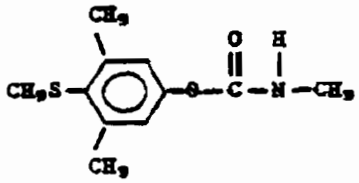
CORE CLASSIFICATION: ACCEPTABLE. This study satisfies the requirements for an acute inhalation study (Guideline Series 81-3).

TOXICITY CATEGORY: II--Warning

A. MATERIALS

1. Test Material

Compound: MESUROL® technical
Chemical name: 3,5-Dimethyl-4-(methylthio) phenol methylcarbamate
Common name: Methiocarb
CAS number: 2032-65-7
Purity of material: 98.8% MESUROL®, active ingredient (a.i.)
Physical description: White crystalline powder
Receipt date: Not reported
Batch number: 861004
Storage conditions: The test material was stored frozen.
Stability: The test material is expected to be stable under the intended conditions of storage and use during the study.
Structural formula:



2. Dose Level:

Test Group

Material: MESUROL® technical

Nominal Concentration (mg/L)	Mean Gravimetric Concentration (mg/L)
2.356	0.199
6.068	0.550
4.139	0.799
8.296	1.443

Controls

Material: Conditioned room air

3. Test Animals

Species: Rats
Strain: Sprague-Dawley CD (SD)BR
Source: Sasco, Inc., Omaha, Nebraska
Receipt date: Not reported
Sex: Male and female
Number: 60 males, 60 females (10/sex/group)
Housing: Individually
Age at exposure: 7-9 Weeks
Weight: 194-279 g (males); 182-255 g (females)

Feed: Purina® Laboratory Rodent Chow #5001 and water ad libitum, except during the exposure period
Assignment: Animals were assigned to groups using a computer-generated randomization procedure

B. TEST PERFORMANCE

Inhalation Chamber

Animals (10/group) were exposed head-only in a 60-liter cylindrical chamber. The animals were positioned in holding tubes (attached near the middle portion of the inhalation chamber) to ensure that the head was exposed to the test atmosphere and the body remained outside of the chamber. Prior to exposure, the inhalation chamber was equilibrated by generating the test atmosphere for at least 30 minutes. The rats were exposed to the four selected test or control (conditioned room air) atmospheres for 4 hours.

Dose Preparation/Generation of Test Atmosphere

The test material was generated as dust using a Wright Dust Feeder. The test material was compressed into the dust feed cup at 1130 psi for all exposure concentrations. Flow of dry, filtered air through the generator was 20 liters/minute. The test material, generated by the dust feeder, was mixed with respirable air in the upper portion of the exposure chamber. The airflow of the exposure chamber, monitored by a rotameter, was recorded approximately every half hour.

Analytical Determinations

At least five gravimetric determinations of the chamber atmosphere concentrations were conducted at each test level. Additional samples were made for exposure concentrations of 0.199 and 0.550 mg/L. Samples were drawn through DM-800 Metricel membrane filters (0.8 µm) from the animals' breathing zone at a rate of 4 Lpm for 0.75-2.25 minutes and the amount of test substance on the filtered paper was determined gravimetrically using a Cahn-27 Automatic Electrobalance. The gravimetric concentration was determined by dividing the mass of the test substance collected on the filter paper by the volume of air sampled.

The nominal concentration of the test material was calculated by dividing the weight loss of the test substance by the total airflow passing through the chamber.

Results of both the gravimetric and nominal concentration analyses are presented below:

Nominal Concentration (mg/L)	Mean Gravimetric Concentration (mg/L)
2.356	0.199
6.068	0.550
4.139	0.799
8.296	1.443

The nominal concentration at each dose level was higher (81-92%) than the corresponding gravimetric concentration, perhaps as a result of losses of the aerosol in the chamber.

Chamber Monitoring

Chamber temperature and chamber relative humidity were recorded continuously throughout exposure. The airflow rates were greater than that required for a turnover rate of at least 10 equivalent chamber volumes per hour. The lowest airflow in this study was 19 Lpm. The chamber temperature ranged from 19.0°C to 22.4°C for the test groups and from 22.0°C to 22.9°C for the control groups. The relative humidity ranged from 55% to 71% for the test groups and from 17% to 66% for the control groups. The relative humidity was lower in the test group chamber in comparison to the control group chamber because test animals were exposed to dry filtered air supplied through the dust feeder and the control animals were exposed to conditioned room air.

Particle-Size Determinations

A TSI Aerodynamic Particle Sizer and Diluter was used to determine the particle-size distribution of the aerosol in the exposure chamber. Samples were analyzed at hourly intervals. Due to time constraints, a sample was not collected during the 0.6-0.8-hour interval at the 0.550 mg/L dose level and at the last collection interval at the 1.443 mg/L dose level. Mass median aerodynamic diameters ranged from 4.0 µm to 5.0 µm and the mean geometric standard deviations ranged from 2.1 to 2.2. (See note below)

Body Weights/Clinical Observations/Gross Necropsies

Animals were weighed prior to treatment on day 0 and on days 3, 7, and 14 (just prior to sacrifice) or at death. The animals were observed for mortality on the day of exposure and for signs of toxicity at approximately 0.1, 0.5, 1.0 and 1.5-3.2 hours postexposure. Detailed physical observation data were recorded at each interval and twice daily (once on weekends) during the 14-day post-exposure period. Gross postmortem examinations were performed on all animals that died during the study as well as those animals that survived until the end of the 14-day postexposure period.

Note: This instrument is a series of pumps with a diluter. The particles pass through a critical orifice and then between two laser beams. 4
If the instrument was overloaded the particle size reported would be larger. As MMAD is between 4 and 5 µm this is only slightly outside over our new requirement (1-4 µm). See Attachment from miles.

Statistics

The LC_{50} and 95% confidence limits were calculated by the probit method.

C. RESULTS AND STUDY AUTHOR'S CONCLUSIONS

Mortality

Inhalation exposure to concentrations of 0.199, 0.550, 0.799 and 1.443 mg/L resulted in mortality rates of 0, 50, 89, and 100%, respectively. No deaths occurred in the control groups or animals dosed at 0.199 mg/L. One female exposed to 0.799 mg/L of the test material died by suffocation. However, this death was not considered to be treatment related. All other deaths were compound related and occurred between days 0 and 4. Based on these findings, the LC_{50} values and 95% confidence limits were calculated as follows:

Sex	LC_{50} (mg/L)*	95% Confidence Limits (mg/L)*
Males	0.585	0.345-0.698
Females	0.433	0.286-0.585

*mg/L of MESUROL® technical

MESUROL® Toxicity

Treatment-related clinical observations in the animals exposed to 0.199, 0.550, and/or 0.799 mg/L consisted of ocular and nasal discharge/staining, salivation, tan oral discharge, hypoactivity, tremors, muscle fasciculations, urine stains, dyspnea, and rales. Other clinical signs noted in the animals dosed at 0.199, 0.550, and 0.799 mg/L included conjunctival swelling and/or alopecia. All animals dosed at 1.443 mg/L died on the day of exposure, therefore no clinical signs were recorded. All signs of toxicity occurred between Day 0 and 6.

Body Weights

Treatment-related body weight decreases were observed in males and females on day 3 postexposure. Surviving animals dosed at 0.199, 0.550, and 0.799 mg/L lost approximately 8.0, 17.3, and 20.9% of their initial body weights, respectively. Most of the control animals gained weight during the study. Body weight recovery occurred during the study, and body weights of all surviving animals were in excess of their pre-exposure body weights by termination of the study.

Gross Necropsy

Treatment-related gross observations included oral, nasal, and ocular staining noted in the animals dosed at 0.199, 0.550, 0.799 and/or 0.1443 mg/L.

D. REVIEWERS' COMMENTS

This study is classified as Core Acceptable.

E. QUALITY ASSURANCE MEASURE

A signed Quality Assurance Statement was presented. The date of the Quality Assurance Statement could not be interpreted from the report provided. A Good Laboratory Practice compliance statement was included.

FINAL

010314

DATA EVALUATION REPORT

MESUROL

Study Type: Mutagenicity: Salmonella typhimurium/Mammalian Microsome Mutagenicity Assay

Prepared for:

Health Effects Division
Office of Pesticide Programs
Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by:

Clement International Corporation
9300 Lee Highway
Fairfax, VA 22031-1207

Principal Reviewer — Kristin Jacobson, MSPH — Date 12/17/92

Independent Reviewer — Nancy E. McCarroll, B.S. — Date 12/17/92

QA/QC Manager — Sharon Segal, Ph.D. — Date 12/18/92

Contract Number: 68D10075
Work Assignment Number: 2-08
Clement Number: 15
Project Officer: James Scott

GUIDELINE §84: MUTAGENICITY
SALMONELLA

MUTAGENICITY STUDIES

12/29/92

EPA Reviewer and Acting Section Head:
Karen Hamernik, Ph.D.
Review Section III, Toxicology Branch I
Health Effects Division (H7509C)

Signature: Karen Hamernik
Date: 5/6/93

DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity: Salmonella typhimurium/mammalian microsome mutagenicity assay

EPA IDENTIFICATION Numbers:

PC Code: 100501

TOX Chem. Number: 578B

MRID Number: 405081-01

TEST MATERIAL: H 321

SYNONYMS/CAS NUMBER: Mesurol; 3,5-dimethyl-4-(methylthio)phenol, methyl-carbamate; mercaptodimethur; metmarcapturon; methicarb/2032-65-7

SPONSOR: Mobay Corporation, Stilwell, KS

STUDY NUMBER: Laboratory project ID/Report number: 91775

TESTING FACILITY: Bayer AG, Wuppertal-Elberfeld, Germany

TITLE OF REPORT: H 321 c.n. Mercaptodimethur. Salmonella/Microsome Test to Evaluate for Point Mutagenic Effect

AUTHOR: Herbold, B.

REPORT ISSUED: January 10, 1986

CONCLUSIONS--EXECUTIVE SUMMARY: In three independently performed Salmonella typhimurium/mammalian microsome plate incorporation assays with tester strains TA1535, TA1537, TA98, and TA100, doses of H 321 ranging from 62.5 µg/plate to 1000 µg/plate in the absence of S9 and from 125 µg/plate to 2000 µg/plate in the presence of S9 were not mutagenic. Compound precipitation was observed at the highest dose tested (12,500 µg/plate +/- S9); doses ≥1000 µg/plate -S9 and ≥2000 µg/plate +S9 were cytotoxic. Although technical concerns were raised (see Reviewers' Comments), these deviations were judged not to have affected the overall study results, therefore, we conclude that H 321 was evaluated over an appropriate range of concentrations and was not mutagenic in this bacterial test system.

STUDY CLASSIFICATION: Acceptable. The study satisfies Guideline requirements (§84-2) for genetic effects Category I, Gene Mutations.

A. MATERIALS:

1. Test Material: H 321

Description: Not provided; however, the chemical structure was included in the report
Identification number: Batch number 234 402 701
Purity: 98.4%
Receipt date: Not reported
Stability: Reported to be stable during the test period and stable in the solvent
Contaminants: None listed
Solvent used: Dimethyl sulfoxide (DMSO)
Other provided information: Neither storage conditions for the test material nor frequency of dosing solution preparation were reported. Analytical determinations to verify test concentrations were not performed.

2. Control Materials:

Solvent/final concentration: DMSO, concentration not stated

Positive:

Nonactivation:		
Sodium azide	10.0 µg/plate	TA1535
4-Nitro-o-phenylenediamine (4-NPA)	0.5 µg/plate	TA1537, TA98
Nitrofurantoin	0.2 µg/plate	TA100
Activation:		
2-Aminoanthracene (2AA)	3 µg/plate	all strains

3. Activation: S9 derived from 200- to 300-g male Sprague-Dawley

x Aroclor 1254	x induced	x rat	x liver
___ phenobarbital	___ noninduced	___ mouse	___ lung
___ none		___ hamster	___ other
___ other		___ other	

The rat liver S9 homogenate was prepared by the testing laboratory and stored at -80°C until use. The S9 mix was prepared immediately prior to use as follows:

Component:	Amount/100 mL
Phosphate buffer (pH unspecified)	100.0 mM
Glucose 6-phosphate	179.1 mg
NADP	315.0 mg
$MgCl_2$	162.6 mg
KCl	246.0 mg
S9	30% (first and third assays) 10% (second assay)

4. Test Organism Used: S. typhimurium strains
____ TA97 _x_ TA98 _x_ TA100 ____ TA102 ____ TA104
_x_ TA1535 _x_ TA1537 ____ TA1538
list any others:

Test organisms were properly maintained? Yes
Checked for appropriate genetic markers (rfa mutation, R factor)? Yes

5. Test Compound Concentrations Used: Similar procedures were used in the three complete and two retest mutation assays that were conducted. In each assay, four plates per dose, per condition, per strain were prepared; all four tester strains were used. In addition, duplicate plates per dose, per strain were prepared to determine the total bacterial population surviving exposure to the varying doses of the test material. Concentrations used in each assay were as follows:

(a) First mutation assay: Five concentrations (20, 100, 500, 2500, and 12,500 µg/plate) were assayed in the presence or absence of S9 mix containing 30% S9.

(b) Second mutation assay: Concentrations of 125, 250, 500, 1000 and 2000 µg/plate, with or without S9 mix containing 10% S9, were used.

(c) Third mutation assay: The concentrations of the test material without metabolic activation were 62.5, 125, 250, 500 and 1000 µg/plate; the S9-activated phase of testing (with S9 mix containing 30% S9) was conducted with concentrations similar to those tested in the second mutation assay.

(d) Retest: Two separate tests, performed on the same day with tester strain TA1537, were conducted to evaluate nonactivated test material concentrations of 62.5, 125, 250, 500 and 1000 µg/plate.

B. TEST PERFORMANCE:

1. Type of Salmonella Assay:
_x_ Standard plate test
___ Pre-incubation (_____) minutes
___ "Prival" modification
___ Spot test
___ Other (describe)

2. Mutation assays: Detailed information on performance of the mutation assays was not provided. The study author indicated, however, that the assay was conducted in accordance with Ames et al. (1973)[1] and (1975)[2]. Plates were incubated at 37°C for 48 hours and scored for revertant colonies; means and standard deviations were determined from the counts of four plates per dose, per condition, per strain.

3. Evaluation Criteria: The test material was considered positive if a consistent dose-related ≥2-fold increase in revertant colonies was observed in at least one tester strain.

4. Protocol: Not provided

C. REPORTED RESULTS:

Mutation Assays:

1. First assay: For the selected doses, ranging from 20 µg/plate to 12,500 µg/plate +/- S9 (30% S9 in the S9 mix), compound precipitation was observed at 12,500 µg/plate +/- S9, and doses ≥2500 µg/plate were severely cytotoxic in all four tester strains. Increased revertant colonies (>2-fold over the respective control) were reported by the study author for tester strain TA1537 at both 100 and 500 µg/plate -S9. However, since the number of colonies observed in the nonactivated solvent control group (3±3 revertants per plate) was low and the mean number of revertants of TA1537 were well within the expected control ranges (7 revertants/plate at each dose level), these increases were not considered biologically significant by our reviewers. No increases in revertant colonies were noted in any other tester strains. Our reviewers did not select results from this assay to present in tabular form because of the relative wide concentration range that was tested and also because of the high concentration of S9 (30%) in the S9 cofactor mix. Although our reviewers noted the marginal response of strains TA1537 and TA98 to the nonactivated positive control 4-NPA, we do not believe that the outcome of the study was affected. The selected concentration of 4-NPA (0.5 µg/plate) approached the limit of detection of this mutagen by strain TA98 (generally levels of 3-5 µg/plate are used to confirm assay sensitivity)[3] and 4-NPA is not a conventional diagnostic mutagen for strain TA1537. Additionally, both strains responded as expected to the S9-activated positive control (3 µg/plate 2AA).

2. Second Assay: Based on the cytotoxicity observed in the first assay, doses for the second assay were adjusted to 125-2000 µg/plate +/- S9.

---

[1]Ames, B.N., W.E. Durston, E. Yamasaki, and F.D. Lee. (1973) Carcinogens are mutagens: A simple test combining liver homogenates for activation and bacteria for detection. Proc Nat Acad Sci 70:2281-2285.

[2]Ames, B.N., J. McCann, and E. Yamasaki. (1975) Methods for detecting carcinogens and mutagens with the Salmonella/mammalian microsome mutagenicity test. Mutat Res 31:347-364.

[3]Haworth, S., T. Lawlor, K. Mortelmans, et al. (1983) Salmonella mutagenicity test results for 250 chemicals. Envir Mutagenesis 5:18.

Representative results from this trial (with 10% S9) are presented in Table 1. Severe cytotoxicity was observed at ≥1000 µg/plate -S9 for tester strains TA 1535, TA1537 and TA98, and in strain TA98 in the presence of S9 activation; at 2000 µg/plate +/- S9, all cultures showed severe cytotoxicity. There were, however, no appreciable increases in revertants at any dose level in any strain.

3. Third Assay: In the third trial, the dose range for the nonactivated phase of testing was adjusted to 62.5-1000 µg/plate. In the presence of S9 activation (30% S9 in the S9-cofactor mix), cultures were exposed to a range of H 321 doses comparable to the levels evaluated in Trial 2. In contrast to the results from the second assay, the highest nonactivated (1000 µg/plate) and S9-activated (2000 µg/plate) doses were either not cytotoxic or cytotoxic only in strain TA1537 in the plate incorporation assay, respectively (Table 2). The accompanying cell titer data did suggest, however, that doses ≥1000 µg/plate were cytotoxic. We conclude, therefore, that the lack of agreement relative to cytotoxicity may have resulted from a minor dilution or plating error. Nevertheless, there was agreement among all assays that nonactivated or S9-activated H 321 was not mutagenic. Although our reviewers concluded that H 321 was not mutagenic in the third trial, the background revertant colony counts for strains TA1535 and TA98 in the presence of 30% S9 were unusually high.

4. Retests with Strain TA1537: Owing to the "increase in mutant counts to over double those of the respective negative control," the nonactivated phase of testing with this strain was repeated twice on the same day with five doses (62.5-1000 µg/plate). The "doubling" for strain TA1537 reported by the study author in the first trial was not reproducible.

In contrast to the negative results with the test material, the positive controls generally induced the expected response in the appropriate tester strain. The use of 4-NPA as the nonactivated positive control for strains TA1537 and TA98 was previously discussed (see Reported Results, First Assay, Section C.1). We assess, therefore, that the overall findings from the positive controls provided sufficient evidence to ensure that the sensitivity of the test system to detect mutagens was adequate.

Based on the above findings, the study author concluded that H 321 was not mutagenic in this bacterial test system.

D. REVIEWERS' DISCUSSION/CONCLUSIONS: We assess that while the individual assays conducted with H 321 were generally not without flaws, collectively, they provided convincing evidence that H 321 was assayed over appropriate concentration ranges in the absence and presence of S9 activation (10 and 30% S9) but failed to induce a mutagenic response. The technical concerns that have been discussed (i.e., use of 4-NPA as the nonactivated positive control for TA1537 and TA98; high background counts for strains TA1535 and TA98 -- Trial 3; and lack of clear cytotoxicity at the highest assayed level in Trial 3) did not affect the overall outcome of the study. We

conclude, therefore, that H 321 was not mutagenic in this series of microbial mutation assays.

E. QUALITY ASSURANCE MEASURES: Was the test performed under GLP? Yes. (A quality assurance statement was signed and dated September 1, 1986.)

F. CBI APPENDICES: Appendix A, Materials and Methods, CBI pp. 7-13.

CORE CLASSIFICATION: Acceptable; the study satisfies the data Guideline requirement (§84-2) for genetic effects Category I, Gene Mutations.

Table 1: Representative Results of the Second Salmonella typhimurium/Mammalian Microsome Mutation Assay with H 321

Substance	Dose/Plate	S9 Activation[a]	Revertants per Plate of Bacterial Tester Strain[b] TA1535	TA1537	TA98	TA100
Solvent Control						
Dimethyl sulfoxide	NR[c]	-	19 ± 7	9 ± 4	23 ± 3	81 ± 17
	NR	+	14 ± 4	11 ± 4	39 ± 4	97 ± 6
Positive Controls						
Sodium azide	10.0 µg	-	961 ± 20	--	--	--
4-Nitro-o-phenylene diamine	0.5 µg	-	--	17 ± 4	78 ± 5	--
Nitrofurantoin	0.2 µg	-	--	--	--	402 ± 26
2-Aminoanthracene	3 µg	+	520 ± 35	382 ± 20	1371 ± 103	2305 ± 496
Test Material						
H 321	500 µg[d]	-	18 ± 3	8 ± 3	24 ± 4	87 ± 5
	1000 µg[e]	-	--	--	--	57 ± 6
	500 µg[d]	+	14 ± 4	6 ± 2	22 ± 5	102 ± 11
	1000 µg[e]	+	9 ± 4	10 ± 2	--	95 ± 8

[a]The S9 mix contained 10% S9
[b]Means and standard deviations of the counts from four plates
[c]NR - Not reported
[d]Results for lower doses (125 and 250 µg/plate +/-S9) did not suggest a mutagenic response.
[e]Higher levels (≥1000 µg/plate -S9 for TA1535, TA1537 and TA98; ≥1000 µg/plate +S9 for TA98; ≥2000 µg/plate -S9 for TA100; and ≥2000 µg/plate +S9 for TA1535, TA1537, and TA100 +S9) were severely cytotoxic.

Note: Data were extracted from Tables 5-8, CBI pp. 20-23.

Table 2: Representative Results of the Third Salmonella typhimurium/Mammalian Microsome Mutation Assay with H 321

Substance	Dose/Plate	S9 Activation[a]	Revertants per Plate of Bacterial Tester Strain[b] TA1535	TA1537	TA98	TA100
Solvent Control						
Dimethyl sulfoxide	NR[c]	-	17 ± 3	5 ± 3	14 ± 3	82 ± 12
	NR	+	62 ± 6	8 ± 1	72 ± 4	118 ± 12
Positive Controls						
Sodium azide	10.0 µg	-	1055 ± 21	--	--	--
4-Nitro-o-phenylene diamine	0.5 µg	-	--	50 ± 13	47 ± 7	--
Nitrofurantoin	0.2 µg	-	--	--	--	319 ± 18
2-Aminoanthracene	3 µg	+	130 ± 19	49 ± 4	264 ± 13	380 ± 34
Test Material						
H 321	500 µg[d]	-	23 ± 3	7 ± 2	12 ± 5	78 ± 6
	1000 µg[e]	-	18 ± 5	5 ± 2	6 ± 1	80 ± 21
	500 µg[d]	+	57 ± 1	6 ± 2	55 ± 10	67 ± 10
	1000 µg	+	51 ± 8	4 ± 2	53 ± 2	101 ± 25
	2000 µg[e]	+	59 ± 4	--	58 ± 6	97 ± 29

[a]The S9 mix contained 30% S9
[b]Means and standard deviations of the counts from four plates
[c]NR = Not reported
[d]Results for lower doses (62.5, 125 and 250 µg/plate -S9; 125 and 250 µg/plate +S9) did not suggest a mutagenic response.
[e]Highest dose tested

Note: Data were extracted from Tables 9-12, CBI pp. 24-27.

TABLE 2

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SALMONELLA

APPENDIX A

MATERIALS AND METHODS
CBI pp. 7-13

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FINAL

010314

DATA EVALUATION REPORT

MESUROL

Study Type: Mutagenicity: Unscheduled DNA Synthesis Assay in Primary Rat Hepatocytes

Prepared for:

Health Effects Division
Office of Pesticide Programs
Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by:

Clement International Corporation
9300 Lee Highway
Fairfax, VA 22031-1207

Principal Reviewer Lynne T. Haber, Ph.D. Date 12/23/92

Independent Reviewer Nancy E. McCarroll, B.S. Date 12/23/92

QA/QC Manager Sharon Segal, Ph.D. Date 12/23/92

Contract Number: 68D10075
Work Assignment Number: 2-08
Clement Number: 16
Project Officer: James Scott

GUIDELINE SERIES 84: MUTAGENICITY
UDS

MUTAGENICITY STUDIES

EPA Reviewer and Acting Section Head:
Karen Hamernik, Ph.D.
Review Section III,
Toxicology Branch I/HED (H7509C)

Signature: [signature] 01-12-93

Date: [signature] 5/6/93

DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity: In vitro unscheduled DNA synthesis assay in primary rat hepatocytes.

EPA IDENTIFICATION Numbers:

PC Code: 100501

Tox Chem. Number: 578B

MRID Number: 407008-01

TEST MATERIAL: Mesurol®

SYNONYMS/CAS NUMBER: 3,5-Dimethyl-4-(methylthio)phenol methylcarbamate; methiocarb; metmarcapturon; mercaptodimethur/2032-65-7

SPONSOR: Mobay Chemical Corp., Stilwell, KS

STUDY NUMBERS: Study report number: 96708; Laboratory study number: T5391.380; Toxicology report number: 1007

TESTING FACILITY: Microbiological Associates, Inc., Bethesda and Rockville, MD

TITLE OF REPORT: Unscheduled DNA Synthesis in Rat Primary Hepatocytes, Test Article Mesurol, Lot No. 861004

AUTHOR: Curren, R. D.

REPORT ISSUED: June 1, 1988

CONCLUSIONS-EXECUTIVE SUMMARY: Mesurol technical was evaluated in two independently performed assays for the potential to cause unscheduled DNA synthesis (UDS) in primary rat hepatocytes; cells from a female rat were used in Trial 1 and hepatocytes from a male rat in Trial 2. Results indicate that the test material was not genotoxic over concentration ranges (1.0-45 µg/mL in Trial 1 and 3.0-60 µg/mL in Trial 2) that included moderately cytotoxic levels. Higher doses (≥60 µg/mL in Trial 1 and 100 µg/mL in Trial 2) were severely cytotoxic. Based on these findings, it was concluded that mesurol technical was tested over an appropriate range of concentrations with appropriate controls and showed no evidence of UDS.

STUDY CLASSIFICATION: Acceptable; the study satisfies Guideline requirements (§84-4) for genetic effects Category III, Other Mutagenic Mechanisms.

A. MATERIALS:

1. Test Material: Mesurol technical

   Description: White powder
   Lot number: 861004
   Purity: 98.8%
   Receipt date: March 4, 1987
   Stability: Reported to be stable
   Contaminants: None listed
   Solvent used: Dimethyl sulfoxide (DMSO)
   Other provided information: The test material was stored at -20°C; test material solutions were prepared immediately prior to use. Analytical determinations were not performed on dosing solutions.

2. Indicator Cells: Primary rat hepatocytes were obtained by the in situ perfusion of the liver of an adult female (Trial 1) or adult male (Trial 2) Sprague Dawley rat, purchased from Charles River Laboratories, Inc. (city not specified).

3. Control Substances: DMSO (10 µL/mL) was the solvent control and untreated cells served as the negative control. 7,12-Dimethylbenz(a)-anthracene (DMBA), prepared in DMSO, was used as the positive control at 3 and 10 µg/mL in both assays.

4. Medium: WME: Williams' Medium E containing 2 mM L-glutamine and gentamicin; WME+: Williams' Medium E with 10% fetal bovine serum.

5. Test Compound Concentrations Used:

   (a) Preliminary cytotoxicity assay: Ten doses (0.3, 0.9, 3.0, 9.0, 30, 90, 300, 900, 3000, and 5000 µg/mL) were assayed.

   (b) UDS assays:

   - Concentrations assayed: Nine doses (0.1, 0.3, 1.0, 3.0, 10, 30, 45, 60 and 100 µg/mL) were assayed in both trials.
   - Concentrations scored: Five doses were scored in each trial. In Trial 1, cells exposed to 1.0, 3.0, 10, 30, and 45 µg/mL were scored, while hepatocytes exposed to 3.0, 10, 30, 45, and 60 µg/mL were scored in Trial 2.

B. STUDY DESIGN:

1. Cell Preparation:

(a) Perfusion technique: Animals were anesthetized with metofane (methoxyflurane) and livers were perfused with Hanks' balanced salt solution containing 0.5 mM EGTA and Hepes buffer, pH 7.3, and with WME containing 80-100 units/ml collagenase and Hepes buffer pH 7.3. The livers were excised and placed in WME-collagenase medium; cells were detached by combing or passage through a sieve.

(b) Hepatocyte harvest/culture preparation: Recovered cells were collected, counted, and seeded in WME+ at a density of $\approx 5 \times 10^5$ cells, either into preconditioned 35-mm tissue culture dishes for the cytotoxicity assay, or onto coverslips in 35-mm tissue culture plates for the UDS assay. Cultures were placed in an incubator for 90-120 minutes (cytotoxicity assay) or 100-165 minutes (UDS assay), washed and fed WME.

2. Preliminary Cytotoxicity Assay: Prepared primary hepatocyte cultures (in duplicate) were exposed to the selected doses of the test compound, negative control (WME), or solvent control (DMSO) for 18-20 hours. Following exposure, an aliquot of culture fluid was removed, centrifuged, and the level of lactic acid dehydrogenase (LDH) activity was measured. Relative cytotoxicity was assessed by subtracting the LDH activity of the solvent control from the LDH activity in the treated cultures and comparing the values to the amount of LDH released by exposure of the solvent control cultures to 1% Triton.

3. UDS Assay:

(a) Treatment: Three hepatocyte cultures on coverslips were fed WME containing 10 µCi/mL [^{3}H] thymidine and exposed for 18-20 hours to the selected test material doses, the negative control (WME), the solvent control (DMSO), or the positive control (DMBA). Treated hepatocytes attached to coverslips were washed, swollen in 1% sodium citrate, fixed in ethanol-acetic acid, dried and mounted. The parallel cytotoxicity assessment was conducted as previously described; three cultures per dose were used.

(b) Preparation of autoradiographs/grain development: Slides were coated with Kodak NTB emulsion, dried, stored for 9-10 days at 4°C in light-tight desiccator boxes, developed in Kodak D-19 developer, fixed, stained with hematoxylin-sodium acetate-eosin, coded and counted.

(c) Grain counting: The nuclear grains of 25 cells randomly chosen from each of three coverslips per treatment were counted. Cytoplasmic background counts were determined by counting three nuclear-sized areas adjacent to the nucleus. Net nuclear grain counts were determined by subtracting the mean cytoplasmic

background count from the nuclear grain count. Nuclei exhibiting toxic effects of treatment, such as uneven staining, disrupted membranes, or irregular shape, were not counted.

4. Evaluation Criteria:

(a) Assay validity: The assay was considered valid if (a) the positive control increased the net nuclear grain count by at least five counts over the control, (b) less than 15% of the cells in the negative control were in repair, and (c) the net nuclear grain count of the solvent control was not more than two standard deviations higher than the net nuclear grain count of the negative control.

(b) Positive response: The assay was considered positive if the test compound induced a dose-related response, with the mean net nuclear grain count increased by at least five counts over the control for at least one dose. In the absence of a dose-related response, the test was also considered positive if the mean net nuclear grain count increased by at least five in at least two successive doses.

5. Protocol: See Appendix B

C. REPORTED RESULTS:

1. Preliminary Cytotoxicity Assay: The cytotoxicity assay was conducted with 10 doses ranging from 0.3 to 5000 µg/mL. Precipitation of the test material was reported at concentrations ≥900 µg/mL. As shown in Table 1, no appreciable cytotoxicity was observed at doses ≤30 µg/mL, while relative cytotoxicity at doses ≥90 µg/mL was ≥90%. Microscopic examination of the monolayers indicated severe cytotoxic effects at concentrations ≥90 µg/mL. Based on these results, 100 µg/mL was selected as the highest dose for the UDS assay.

2. UDS Assay: The study author stated that the UDS assay was repeated "several times" for technical reasons. It is not clear if this statement refers only to the two assays for which data were reported, or if additional assays were performed. The first trial was conducted with hepatocytes from a female rat, and tested nine levels of mesurol technical, ranging from 0.1 to 100 µg/mL. As shown in Table 2, relative cytotoxicity was dose related, and ranged from 11% at 30 µg/mL to 93% at 100 µg/mL. Concentrations ≤10 µg/mL were not cytotoxic. Microscopic evaluation of the cells revealed cytotoxic effects at levels ≥30 µg/mL, slight cytotoxicity at 10 µg/mL, and normal cellular morphology at lower doses. Cultures exposed to the two highest doses (60 and 100 µg/mL) were not scored because of excessive cytotoxicity. As further shown in Table 2, there was no evidence of genotoxicity at any assayed level.

TABLE 1. Representative Results of the Preliminary Cytotoxicity Assay with Mesurol Technical: Lactic Acid Dehydrogenase (LDH) Activity

Treatment	Dose	Average[a] LDH Activity (Units/L)	Corrected[b] LDH Activity (Units/L)	Relative Cytotoxicity[c] (%)
Negative Control				
Culture medium	--	83.0	-9.0	-2
Solvent Control				
Dimethyl sulfoxide	10 µL/mL	92.0	0.0	0
Dimethyl sulfoxide +1% Triton	10 µL/mL	480.0	388.0	100
Test Compound Control				
Mesurol technical +1% Triton	5000 µg/mL	522.5	430.5	111
Test Compound				
Mesurol technical	9.0 µg/mL[d]	103.0	11.0	3
	30 µg/mL	120.5	28.5	7
	90 µg/mL	443.0	351.0	90
	300 µg/mL[e]	463.0	371.0	96

[a]Average of two samples

[b]Corrected LDH = Average LDH - Solvent Control LDH

[c]Relative Cytotoxicity = $\frac{\text{Corrected LDH}}{\text{Corrected LDH of Solvent Control + 1\% Triton}} \times 100\%$

[d]Lower doses (0.3, 0.9, and 3.0 µg/mL) showed no evidence of cytotoxicity.

[e]Higher doses (900, 3000, and 5000 µg/mL) were severely cytotoxic and insoluble.

Note: Data were extracted from the study report; see CBI p. 14.

TABLE 2. Representative Results of the Initial Unscheduled DNA Synthesis Rat Hepatocyte Assay with Mesurol Technical[a]

Treatment	Dose	Cytotoxicity: Average[b] LDH Activity (units/L)	Cytotoxicity: Corrected[c] LDH Activity (units/L)	Cytotoxicity: Relative Cytotoxicity[d] (%)	UDS Activity: No. of Cells Scored	UDS Activity: Mean Net Nuclear Grain Count ± S.D.[e]	UDS Activity: Percent Cells with ≥5 Grains
Negative Control							
Culture medium	--	28.3	-2.0	-1	75	-1.2±1.2	0
Solvent Controls							
Dimethyl sulfoxide	10 µL/mL	30.3	0.0	0	75	-1.1±1.1	0
Dimethyl sulfoxide +1% Triton	10 µL/mL	333.7	303.3	100	--	--	--
Test Compound Control							
Mesurol technical +1% Triton	100 µg/mL	320.3	290.0	96	--	--	--
Positive Control							
7,12-Dimethylbenz(a)anthracene[f]	3 µg/mL	65.0	34.7	11	75	15.6±7.0[g]	96
Test Compound							
Mesurol technical	10 µg/mL[h]	31.3	1.0	0	75	-1.4±1.2	0
	30 µg/mL	65.0	34.7	11	75	-0.5±1.2	0
	45 µg/mL	198.0	167.7	55	75	0.2±2.2	3
	60 µg/mL[i]	244.7	214.3	71	--	Cytotoxic	

[a]Performed with hepatocytes recovered from a female rat
[b]Average of three samples
[c]Corrected LDH = Average LDH - Solvent Control LDH

[d]Relative Cytotoxicity = $\frac{\text{Corrected LDH}}{\text{Corrected LDH of Solvent Control + 1\% Triton}} \times 100\%$

[e]Means and standard deviations from the counts of 25 cells/culture.
[f]Positive results were also obtained with 10.0 µg/mL.
[g]Fulfills the reporting laboratory's criterion for a positive response (i.e., ≥5 net nuclear grains)
[h]Results for lower doses (1.0, and 3.0 µg/mL) did not suggest a genotoxic or cytotoxic effect.
[i]The highest assayed concentration (100 µg/mL) was severely cytotoxic.

Note: Data were extracted from the study report; see CBI pp. 15 and 16.

The second assay was conducted with hepatocytes from a male rat, over a comparable concentration range (0.1-100 µg/mL). Results were in general agreement with the findings from Trial 1, although the test compound was slightly less cytotoxic to the hepatocytes recovered from the male rat (Table 3). There was, however, no evidence of genotoxicity at any level scored for UDS (3.0-60 µg/mL). By contrast, the positive control (DMBA at 3.0 and 10.0 µg/mL) induced a marked increase in UDS. Based on these findings, the study author concluded that mesurol technical was negative in the primary rat hepatocyte UDS assay.

D. REVIEWERS' DISCUSSION/CONCLUSIONS: We assess that the study author's interpretation of the data was correct. In a well-conducted assay, mesurol technical was tested to cytotoxic doses but failed to induce UDS. The response of the test system to the positive control indicated that the assay was sufficiently sensitive to detect a mutagenic response. We, therefore, conclude that mesurol technical was not genotoxic in this test system.

E. QUALITY ASSURANCE MEASURES: Was the test performed under GLPs? Yes. (A quality assurance statement was signed and dated June 3, 1988.)

F. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 8-11; Appendix B, Protocol and Protocol Amendment, CBI pp. 20-29.

CORE CLASSIFICATION: Acceptable; the study satisfies Guideline requirements (§84-4) for genetic effects Category III, Other Mutagenic Mechanisms.

TABLE 3. Representative Results of the Confirmatory Unscheduled DNA Synthesis Rat Hepatocyte Assay with Mesurol Technical[a]

Treatment	Dose	Cytotoxicity: Average[b] LDH Activity (units/L)	Cytotoxicity: Corrected[c] LDH Activity (units/L)	Cytotoxicity: Relative Cytotoxicity[d] (%)	UDS Activity: No. of Cells Scored	UDS Activity: Mean Net Nuclear Grain Count ± S.D.[e]	UDS Activity: Percent Cells with ≥5 Grains
Negative Control							
Culture medium	--	73.3	-12.0	-5	75	-0.4±1.5	0
Solvent Controls							
Dimethyl sulfoxide	10 µL/mL	85.3	0.0	0	75	0.3±1.9	0
Dimethyl sulfoxide +1% Triton	10 µL/mL	346.0	260.7	100	--	--	--
Test Compound Control							
Mesurol technical +1% Triton	100 µg/mL	498.0	412.7	158	--	--	--
Positive Control							
7,12-Dimethylbenz(a)anthracene[f]	3 µg/mL	106.3	21.0	8	75	10.7±4.1[g]	93
Test Compound							
Mesurol technical	30 µg/mL[h]	121.0	35.7	14	75	-0.8±1.6	0
	45 µg/mL	176.7	91.3	35	75	-1.5±1.7	0
	60 µg/mL	236.7	151.3	58	75	-0.6±1.8	0
	100 µg/mL	430.7	345.3	132	--	Cytotoxic	

[a]Performed with hepatocytes recovered from a male rat
[b]Average of three samples
[c]Corrected LDH = Average LDH - Solvent Control LDH

[d]Relative Cytotoxicity = $\frac{\text{Corrected LDH}}{\text{Corrected LDH of Solvent Control + 1\% Triton}} \times 100\%$

[e]Means and standard deviations from the counts of 25 cells/culture.
[f]Positive results were also obtained with 10.0 µg/mL.
[g]Fulfills the reporting laboratory's criterion for a positive response (i.e., ≥5 net nuclear grains)
[h]Results for lower doses (3.0 and 10 µg/mL) did not suggest a genotoxic or cytotoxic effect.

Note: Data were extracted from the study report; see CBI pp. 17 and 18.

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